Functional Cell-Based Assays
To Identify Neutralizing Anti-Drug Antibodies

Assay Principle and Validation
Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active β-gal. This then catalyzes the formation chemiluminescent products providing a highly amplified signal, and thus an assay technology of high sensitivity.
By exploiting the natural biology of various receptors, PathHunter EFC technology can be used to create simple, cell-based chemiluminescent assays. (A) By tagging the receptor and the activation-based intracellular response protein, we can create a specific assay for an activated receptor, e.g. β-Arrestin recruitment to an activated GPCR. (B) By tagging the early endosome and the receptor, we can follow receptor internalization for GPCRs, RTKs and other receptors. (C) By tagging various receptors we can monitor the formation of receptor heterodimers and homodimers. (D) By tagging cellular pathway proteins, signaling events downstream of receptor activation including cAMP accumulation, protein translocation or protein degradation can be measured.
How do we detect Neutralizing ADAs?

**Target-Proximal Response**
- Recruitment of protein upon receptor activation

**Normal Human Serum**
- Light Output

**Human Serum Containing Neutralizing Antibodies**
- Reduced Light

**DRUG**
- Receptor Activation

**Protein**
- Target-Proximal Response
Key Features of PathHunter® Cell-Based Assays for Detection of Neutralizing ADAs

- **Target-specific** functional response
- **High tolerance** of human serum
- **Ease of Transfer**
  - Simple protocol; **Rapid** results
  - Available “Thaw & Use” kit format for assays
- **High sensitivity** for low antibody concentrations
- **High reproducibility**
- Ability to process many samples; **High throughput**
Target-specific Functional Response to Drug

- Harness **native biology** of receptor to create assay
- Receptor activation leads to proximal signaling event inside the cell
- Target-specific signal
  - Tagged receptor
  - Tagged response protein

IGF1R Assay

- Graph showing RLU vs. Concentration (Conc) for different substances:
  - GLP1 [M]
  - Exendin-4 [M]
  - IGF1 [g/ml]
  - hGH [g/ml]
Assay Tolerance of Human Serum

Low Effect on HRH1 Assay

- No serum
- 1% serum
- 2.5% serum
- 5% serum
- 10% serum

Medium Effect on CMKLR1 Assay

- No serum
- 5% serum
- 10% serum
**Simple Protocol, Rapid Results**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Time to results from plating</th>
<th>Time to results from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>96+ hours*</td>
<td>48-72 hours*</td>
</tr>
<tr>
<td>Proliferation</td>
<td>72-96 hours**</td>
<td>48-72 hours**</td>
</tr>
<tr>
<td>Gene Reporter Assays</td>
<td>48-72 hours**</td>
<td>24-48 hours**</td>
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<tr>
<td>PathHunter Assays</td>
<td>24-30 hours</td>
<td>2-4 hours</td>
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</table>

* Fernandez-Salas et al., PLOS ONE. 2012. Botulinum Neurotoxin Serotype a Specific Cell-Based Potency Assay to Replace the Mouse Bioassay
** Herbrand et al., Application Note. 2013. Applicability of TNFa blocker bioassays in a QC environment.
## Day-to-day & Operator Reproducibility

### GLP1R Assay

<table>
<thead>
<tr>
<th></th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Plate 3</th>
<th>Plate 4</th>
<th>Plate 5</th>
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<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Operator 1</strong></td>
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<td></td>
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<tr>
<td>Avg %CV</td>
<td>4.23</td>
<td>4.07</td>
<td>3.97</td>
<td>3.88</td>
<td>3.95</td>
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<td>S:B</td>
<td>29.96</td>
<td>29.44</td>
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<td>31.8</td>
<td>29.9</td>
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<tr>
<td>Avg. EC50</td>
<td>2.43E-09</td>
<td>2.30E-09</td>
<td>2.27E-09</td>
<td>2.43E-09</td>
<td>2.47E-09</td>
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<tr>
<td><strong>Day 2</strong></td>
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</tr>
<tr>
<td><strong>Operator 2</strong></td>
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<tr>
<td>Avg %CV</td>
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<td>3.79</td>
<td>3.77</td>
<td>4.82</td>
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<tr>
<td>S:B</td>
<td>25.48</td>
<td>24.71</td>
<td>24.7</td>
<td>24.8</td>
<td>24.88</td>
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<tr>
<td>Avg. EC50</td>
<td>2.36E-09</td>
<td>2.28E-09</td>
<td>2.20E-09</td>
<td>2.21E-09</td>
<td>2.19E-09</td>
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<tr>
<td><strong>Day 3</strong></td>
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<tr>
<td><strong>Operator 3</strong></td>
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<tr>
<td>Avg %CV</td>
<td>5.31</td>
<td>5.97</td>
<td>7.12</td>
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<tr>
<td>S:B</td>
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<td>13.74</td>
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<td>Avg. EC50</td>
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<td>2.32E-09</td>
<td>2.63E-09</td>
<td>2.52E-09</td>
<td>2.40E-09</td>
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Case Studies
DiscoveRx Assays for Detection of Neutralizing Anti-Drug Antibodies

• Case Study 1:
  • Feasibility study of novel Cytokine analog drug

• Case Study 2:
  • Development and validation of novel GPCR analog drug
Case Study 1: Project Background

Substance: Agonist Drug to Cytokine Receptor
Clinical Phase I and Phase II trials

• Ideal Bioassay for Drug
  • Reflect the native biology of the agonist drug
  • Be sensitive to small volumes in matrix
  • Simple experimental design with throughput
  • High degree of accuracy and reproducibility
Existing Assays and Their Limitations

**Existing Assays**
- Whole Animal Assays
- Cell Proliferation
- Engineered proliferation assay

**Challenges**
- Long, complex assay protocols
  - 4-20 day, multi-step assays
- High variability
- Downstream assay response
  - Assay may not be specific to the receptor
  - Human serum interference
- Low S:B ratio
  - Requires extensive assay development
- Expensive
PathHunter Cytokine Receptor Assay Principle

SH2-domain protein recruitment to activated receptor
Serum Tolerance of PathHunter Assay
Intra and Inter-Assay Precision

**Intra-assay precision**
- Low (CV=7%)
- Mid (CV=7%)
- High (CV=12%)

**Inter-assay precision**
- 25% NHS Exp 1 (EC50 0.023)
- 25% NHS Exp 2 (EC50 0.021)
- 25% NHS Exp 3 (EC50 0.022)
- 25% NHS Exp 4 (EC50 0.039)
- 25% NHS Exp 5 (EC50 0.035)
Summary: Validation of Assay for ADA use

• Currently using our assay
  • Bioassay for drug potency and QC Lot release
  • Bioassay for neutralizing antibody analysis

• Advantages
  • Less complex
  • Faster
  • Highly specific response
  • Better estimated sensitivity
  • Lower variability
  • Cost-effective
## World’s Largest Bioassay Panel for Biologics

### Assays for almost every known GPCR

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Number of Assays</th>
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<tbody>
<tr>
<td>Arrestin Recruitment (Human)</td>
<td>182</td>
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<tr>
<td>Arrestin Recruitment (Ortholog)</td>
<td>77</td>
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<tr>
<td>cAMP</td>
<td>119</td>
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<td>Calcium</td>
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<td>Internalization</td>
<td>92</td>
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<tr>
<td>Orphan GPCRs</td>
<td>82</td>
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<td><strong>TOTAL</strong></td>
<td><strong>&gt;570</strong></td>
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### RTK Targets

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<th>Receptor Tyrosine Kinases</th>
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<tr>
<td>c-KIT</td>
<td>ErbB4</td>
</tr>
<tr>
<td>c-MET</td>
<td>FGFR1</td>
</tr>
<tr>
<td>c-Ret-GFRα2</td>
<td>IGF1R</td>
</tr>
<tr>
<td>DDR1</td>
<td>INSR</td>
</tr>
<tr>
<td>DDR2</td>
<td>INSR</td>
</tr>
<tr>
<td>EphA4</td>
<td>(Internalization)</td>
</tr>
<tr>
<td>EphA5</td>
<td>PDGFRa</td>
</tr>
<tr>
<td>EphA7</td>
<td>PDGFRb</td>
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<tr>
<td>EphB1</td>
<td>TrkA</td>
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<tr>
<td>EphB2</td>
<td>TrkA-P75</td>
</tr>
<tr>
<td>EphB3</td>
<td>TrkB</td>
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<tr>
<td>EphB4</td>
<td>TrkB-P75</td>
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<tr>
<td>ErbB1</td>
<td>TrkC</td>
</tr>
<tr>
<td>ErbB2-ErbB3</td>
<td>TrkC-P75</td>
</tr>
</tbody>
</table>

### Other Receptors

- **Cytokine Receptors**
  - GCSF Receptor
  - Epo Receptor
  - GHR
  - PRLR

- **Other Receptor Assays**
  - TGFβR1&2
  - BMP receptors
  - TNF receptor
  - TLR receptors
  - IL17 receptors
  - Frizzled receptor

### Assays for Largest Collection of Extracellular Ligands

Growth Factors, Cytokines, Hormones, Chemokines, Inflammatory ligands etc.

**Simply Click Here** to request an updated list of available assays

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Detection reagents
Plates